Phosphoinositide 3-kinases: a conserved family of signal transducers

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Phosphoinositide 3-kinases (PI3Ks) generate lipids that are implicated in receptor-stimulated signalling and in the regulation of membrane traffic. Several distinct classes of PI3Ks have now been identified that have been conserved throughout eukaryotic evolution. Potential signalling pathways downstream of PI3Ks have been elucidated and PI3K function is now being characterised in several model organisms.

MEMBRANE LIPIDS DO not only have a structural role, but are also involved in signalling processes. A well-known example of this is the hydrolysis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2]* by phospholipase C (Fig. 1), giving rise to diacylglycerol and inositol(1,4,5)P_3, and to subsequent intracellular Ca^{2+} release and protein kinase C (PKC) activation[1]. The lipids in the PtdIns(4,5)P_2 pathway are also substrates for phosphoinositide 3-kinases (PI3Ks), which phosphorylate the hydroxyl group at position 3 on the inositol ring and induce different signals (Fig. 1). This review will focus on three areas of PI3K research in which substantial progress has recently been made. First, we will describe the structural features and classification of the different PI3Ks. Next, we will review recently identified molecules that act upstream and downstream of PI3Ks. Finally, we will describe work on PI3K in different eukaryotic organisms, and discuss how these studies may contribute to our future understanding of the function of PI3K signalling.

PI3Ks generate three different lipids

PI3Ks convert PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 to PtdIns(3)P, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, respectively (Fig. 1).

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PtdIns(3)P is constitutively present in eukaryotic cells and its levels are largely unaltered upon cellular stimulation. By contrast, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 are almost absent from resting cells. Their intracellular concentration rises sharply upon stimulation with a variety of ligands, suggesting a likely function as second messengers (reviewed in Ref. 2). PI3K lipid products are not substrates for phospholipases, so they are not degraded into soluble inositol phosphates. Instead, phosphatases mediate their catabolism by removing the phosphate group at position 3 or 5 of the inositol ring[2,3] (Fig. 1). Several 5-phosphatase genes have now been cloned (for overview, see Ref. 3) and their role in signalling is being elucidated (for example, see Ref. 4).

PI3Ks fall into three classes

PI3K catalytic subunits can be divided into three main classes on the basis of their *in vitro* lipid substrate specificity, structure and likely mode of regulation (Table I; see also Ref. 5).

Class I PI3Ks phosphorylate PtdIns, PtdIns(4)P and PtdIns(4,5)P_2. *In vivo*, however, their preferred substrate is likely to be PtdIns(4,5)P_2. All mammalian PI3Ks from this class interact with active, GTP-bound Ras (Refs 6–10; R. Wetzker, pers. commun.). They all form heterodimeric complexes with adaptor proteins that link them to different upstream signalling events. Class I PI3K catalytic subunits can be subdivided into two subclasses (A and B) according to the type of adaptor subunit with which they associate.

Class I_A PI3Ks are 110–130 kDa proteins that interact with adaptor subunits containing *src* homology-2 (SH2) domains. These adaptors bind phosphorylated Tyr residues, thereby linking class I_A PI3K catalytic subunits to Tyr kinase signalling pathways. Class I_A PI3K catalytic subunits include mammalian p110α, -β and δ, and homologous molecules from several other species (Table I). They contain several conserved regions including the adaptor- and the Ras-binding sites, the PI-kinase region (PIK; of unknown function, also found in PI4Ks) and the carboxy-terminal kinase domain[5].

To date, eight different adaptor subunits for class I_A catalytic subunits have been described (seven in mammals encoded by three different genes, and one in *Drosophila*; see Fig. 2). They all contain two SH2 domains linked by an inter-SH2 region, which is both necessary and sufficient for binding to the catalytic subunits. The SH2 domains bind phosphorylated Tyr residues specifically within a pTyr-x-x-Met motif. The 85 kDa adaptors also contain an SH3 domain and a breakpoint cluster region (BCR)-homology domain (BH), whose precise binding partners and/or regulatory role are unclear. There has been no report to date of a preferential coupling between any of the class I_A adaptors and catalytic subunits, although it is possible that tissue-specific differences in function or regulation may exist.

Class I_B PI3Ks are stimulated by G-protein βγ subunits, and do not interact with the SH2-domain-containing adaptors that bind to class I_A PI3Ks (Table I). The first identified member of this PI3K subfamily, p110γ (Ref. 11) contains an amino-terminal Ras-binding site, a PIK domain and a catalytic domain (Table I). Stephens and co-workers recently reported the isolation of a regulatory p101 subunit that associates tightly with p110γ (Ref. 12). This novel adaptor does not display any homology with known proteins and the exact mechanism by which it mediates coupling of p110γ to G proteins remains unknown.

Class II PI3Ks are larger (> 200 kDa) enzymes that phosphorylate *in vitro* PtdIns and PtdIns(4)P, but not PtdIns(4,5)P_2. Their defining feature is a C2 domain[13] at their carboxyl terminus (Table I). The C2 domains of class II PI3Ks lack critical Asp residues that coordinate

*Nomenclature used is according to Ref. 1. Phosphoinositide (PI) is used as a generic term, whereas phosphatidylinositol (PtdIns) is used to indicate specific phosphoinositides, e.g. PtdIns(3)P.

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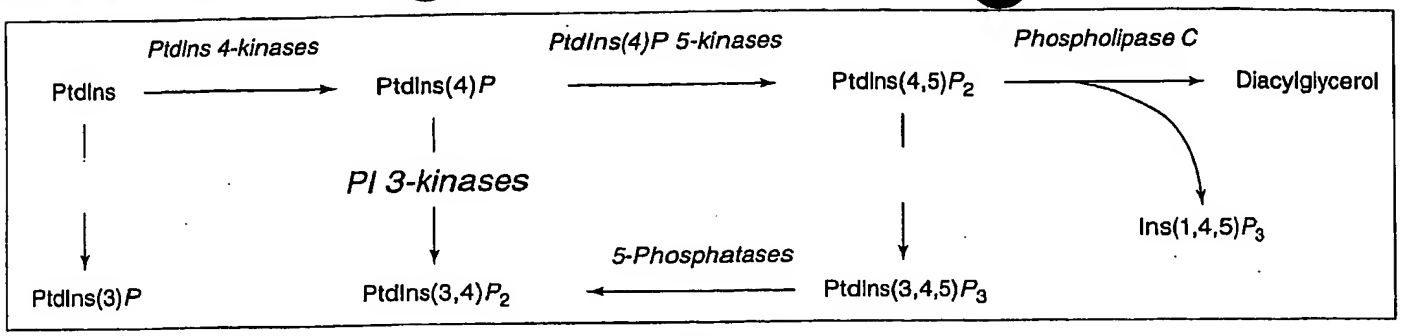


Figure 1
Phosphoinositide pathways. Ins, inositol; PI 3-kinases, phosphoinositide 3-kinases; PtdIns, phosphatidylinositol.

binding of Ca^{2+} in the first C2 domain of synaptotagmin[13]. Consistent with this, a class II PI3K has been found to bind lipids in a Ca^{2+}-independent manner[14]. At present, it is unknown whether class II PI3K activity is regulated by extracellular stimuli.

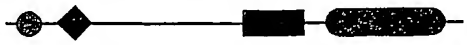
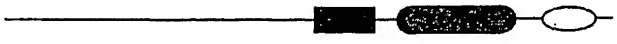
Class III PI3Ks have a substrate specificity restricted to PtdIns. These PI3Ks are homologous to Vps34p, the only PI3K present in yeast. Vps34p is essential for the trafficking of newly formed proteins from the Golgi to the vacuole, the equivalent of the mammalian lysosome (reviewed in Refs 3, 15, 16). Members of this class of PI3Ks also occur as heterodimers. Yeast Vps34p is found in complex with Vps15p, a 170 kDa Ser/Thr kinase, which both activates and recruits Vps34p to membranes[15]. Similarly, human Vps34p associates with a 150 kDa Vps15p homologue[17]. The current hypothesis is that class III PI3Ks and their PtdIns(3)*P* lipid product fulfil a housekeeping role in constitutive membrane trafficking and vesicle morphogenesis[3,15,16]. It is worth mentioning here that class I and II enzymes and their lipid products are also likely to have a function in vesicular trafficking, for example in post-endocytic sorting of ligand-stimulated receptors[3,15,16].

Signalling via PI3Ks

Recent advances in the understanding of signals feeding into and relayed by mammalian class I PI3Ks will be reviewed, focusing on the signalling molecules *per se*, without elaborating on the multiple biological responses (such as cytoskeletal rearrangements, cellular migration, mitogenesis, differentiation and protection from apoptosis) in which PI3Ks have been implicated (reviewed in Refs 18–20).

What happens upstream of class I PI3Ks? Class I PI3Ks are involved in signalling by the majority of receptors with intrinsic or associated (e.g. Src-like) Tyr kinase

Table I. A classification of phosphoinositide 3-kinase (PI3K) family members

Class	*In vitro* lipid substrates and structural features of catalytic subunits[a]	Subunits[b] Catalytic	Subunits[b] 'Adaptor'	Regulation
I	PtdIns, PtdIns(4)*P*, PtdIns(4,5)P_2			
A		p110α, β, δ (m) Dp110 (Dm) AGE-1 (Ce) PIK1, PIK2 (Dd)	p85α, β (m) p55α, γ (m) p50α (m) p60 (Dm)	Tyr kinases and Ras
B		p110γ (m) PIK3 (Dd)	p101 (m)	G protein βγ subunits and Ras
II	PtdIns, PtdIns(4)*P*			
		PI3K-C2α/Cpk-m/p170 (m) PI3K-C2β (m) PI3K_68D/Cpk (Dm) PI3K-C2 (Ce)	?	?
III	PtdIns			
		Vps34p[c]	Vps15p (Sc) p150 (m)	Constitutive?

[a]Key of structural motifs: adaptor-binding (light purple); Ras-binding (green); C2 (yellow); PIK (dark purple); kinase domain (red).
[b]For the proteins other than those derived from yeast, fruit fly and mammals, no biochemical proof of PI3K lipid kinase activity is available. These enzymes have been allocated to a particular class of PI3K mainly based on primary sequence homology of the core kinase domain[5]. The abbreviations used are: m, mammalian; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Sc, *Saccharomyces cerevisiae*. The GenBank/EMBL accession numbers for class I and II catalytic subunits are: *mammalia*: p110α (human: Z29090, HSU79143; mouse: U03279; bovine: M93252), p110β (human: S67334), p110γ (human: X83368; pig: Y10743), p110δ (human: Y10055, U57843, U86587; mouse, U86453), PI3K-C2α (human: Y13367), Cpk-m (also known as p170) (mouse: U52193; U55772), PI3K-C2β (Y13892, Y11312) – *D. melanogaster*: Dp110 (Y09070), PI3K_68D (also known as Cpk) (X92892; U52192) – *C. elegans*: age-1 (U56101), putative C2-domain containing PI3K (on cosmid Z69660) – *D. discoideum*: PIK1 (U23476), PIK2 (U23477) and PIK3 (U23478). Accession numbers for p101, Vps15p and p150 are Y10742, M59835 and Y08991, respectively.
[c]The prototype of the class III PI3Ks is the *S. cerevisiae* protein Vps34p (X53531). Vps34p homologues from other species are not shown individually. They are: human PI3K (Z46973); *D. melanogaster*, PI3K_59F (X99912); *D. discoideum*, PIK5 (U23480); and the Vps34p-related PI3Ks from *Schizosaccharomyces pombe* (U32583), Soybean (L29770), *Arabidopsis thaliana* (U10669) and *C. elegans* (Y12543).

activity, and by receptors linked to heterotrimeric G proteins (for a listing of the signals that trigger class I PI3K activation, see Refs 2, 21).

At present, it is unclear how G-protein signals are relayed from the plasma membrane to the class I_B enzymes. For class I_A PI3Ks, the phosphorylated Tyr residues, generated on receptors or associated substrate molecules (such as IRS-1/2 in signalling by insulin and cytokines) form the docking sites for the SH2 domains of the PI3K adaptor subunits. This adaptor-mediated translocation of PI3Ks to receptor Tyr kinases and their substrates is likely to help position the catalytic subunits close to membranes that contain their lipid substrates.

In addition, class I PI3Ks interact with Ras proteins in a GTP-dependent manner. Thus far, this interaction has only been studied in detail for the p110α–p85α complex[6,8–10]. The Ras-related proteins, Rac (which has been implicated in signalling downstream of PI3Ks[22]) and Rho, do not bind p110α–p85α (Ref. 6). *In vitro*, incubation of GTP–Ras with p110α–p85α results in a modest increase in PI3K kinase activity[8]. Co-expression studies of p110α–p85α with various Ras mutants indicate that Ras can regulate p110α–p85α *in vivo*[6,10] (Fig. 3). Evidence from experiments using platelet-derived growth factor (PDGF)-receptor mutants also suggests that accumulation of GTP-bound Ras is required for full activation of class I_A PI3Ks by PDGF[23]. At present, however, it is not yet known to what extent PI3K activation by receptor Tyr kinases also occurs independently of Ras (Fig. 3).

Taken together, these data indicate that PI3Ks might be another class of Ras effector molecules, alongside proteins such as the Raf Ser/Thr kinases (reviewed in Ref. 24). The interaction of Ras with a PI3K might result in allosteric activation and/or contribute to PI3K recruitment to the plasma membrane. Interestingly, Ras effector mutants have been identified that interact with p110α, but not with Raf-1 (and *vice versa*)[25] consistent with the existence of a Raf-independent signalling pathway downstream of Ras (see also below). It should be noted that data have also been reported that position Ras downstream of PI3Ks[26].

What happens downstream of PI3Ks? It is generally hypothesised that PI3K lipid products interact with certain proteins and modulate their localisation and/or activity. The recent characterisation of protein modules, such as pleckstrin-homology (PH)[27] and C2 domains, which can bind lipids, supports this view. If selective targets downstream of receptor-stimulated PI3Ks exist, they are expected to have very high affinity and specificity for PtdIns(3,4)P_2 and/or PtdIns(3,4,5)P_3 over PtdIns(4,5)P_2 because the latter lipid is estimated to be at least 10–100-times more abundant in most stimulated cells[28]. The observation that certain PH domains seem to bind specifically to either PtdIns(4,5)P_2 or PtdIns(3,4,5)P_3 is consistent with such a hypothesis[29]. However, another important consideration is that several PI3Ks possess intrinsic protein kinase activity, which might be involved in PI3K signalling. Thus far, only auto- and intersubunit phosphorylation of PI3Ks has been documented and no *in vivo* protein kinase substrates for PI3Ks have been identified[9,30].

One postulated function for PI3Ks is in cytoskeletal reorganisation via exchange factors that regulate the small GTP-binding protein Rac[22], and that modulate the affinity of integrins for the extracellular matrix[52]. In addition, several protein Ser/Thr kinases have been placed downstream of PI3Ks in receptor-stimulated signalling, including Akt [also termed protein kinase B (PKB) or RAC-PK (Related to PKA and PKC-protein kinases)], p70 ribosomal S6 kinase ($p70^{S6k}$) and PKC (Fig. 3).

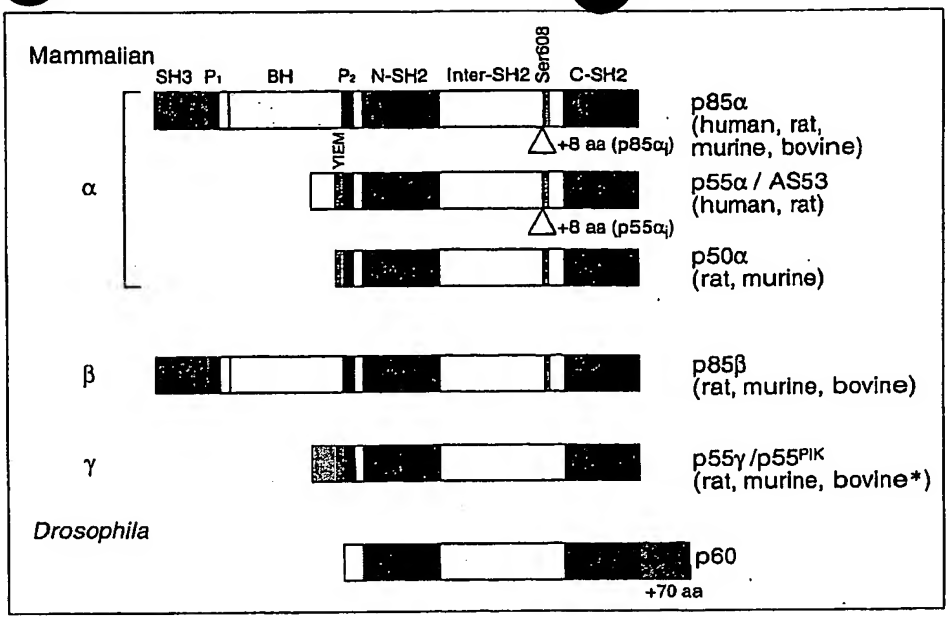


Figure 2

Overview of the different adaptor subunits for class I_A phosphoinositide 3-kinases. P, Pro-rich region; BH, bcr homology region. p50α and p55α (also known as p85/AS53) are splice variants of p85α, whereas p85β and p55γ (also indicated as $p55^{PIK}$) are encoded by different genes. Triangles indicate further splice insertions in p85α and p55α (here named $p85α_i$ and $p55α_i$). Possible regulatory phosphorylation sites are indicated as Ser608 (Ref. 30) and YIEM. GenBank/EMBL accession numbers are: p85α (human, M61906; bovine, M61745; mouse, M60651, U50413; rat, D64045), p50α (mouse, U50414; rat, U50412), p55α (human, U49349; rat, D64048), p85β (bovine, M61746; rat, D64046), p55γ (mouse, S79169; rat, D64047), p60 (*Drosophila melanogaster*, Y12498). *Bovine p55γ (M. D. Waterfield *et al.*, unpublished).

(1) Akt and its target GSK3. The Akt Ser/Thr protein kinases, the cellular homologues of the retroviral oncogene *v-akt*, are activated upon receptor-Tyr kinase stimulation (reviewed in Ref. 31). The three mammalian Akt molecules identified to date are all composed of an amino-terminal PH domain, followed by a catalytic domain and a small (~70 amino acids) carboxy-terminal extension, which lacks sequence homology to other proteins.

The activation of Akt most likely involves specific phosphorylations as well as a PH-domain mediated lipid binding. However, while it is clear that Akt can bind lipids, the specificity of this event and its effect on the activity and on the intracellular localisation of Akt remain controversial[31,32]. Upon stimulation of cells with insulin, two sites in Akt-1 (Thr308 in the catalytic domain and Ser473 in the carboxy-terminal tail) become phosphorylated *in vivo* in a PI3K-sensitive manner[33] (Fig. 3). Phosphorylation of both residues appears to be critical for high-level activity of Akt, suggesting that one or more upstream kinases activate Akt *in vivo*. Recently, a protein kinase activity has been purified which phosphorylates Akt at Thr308

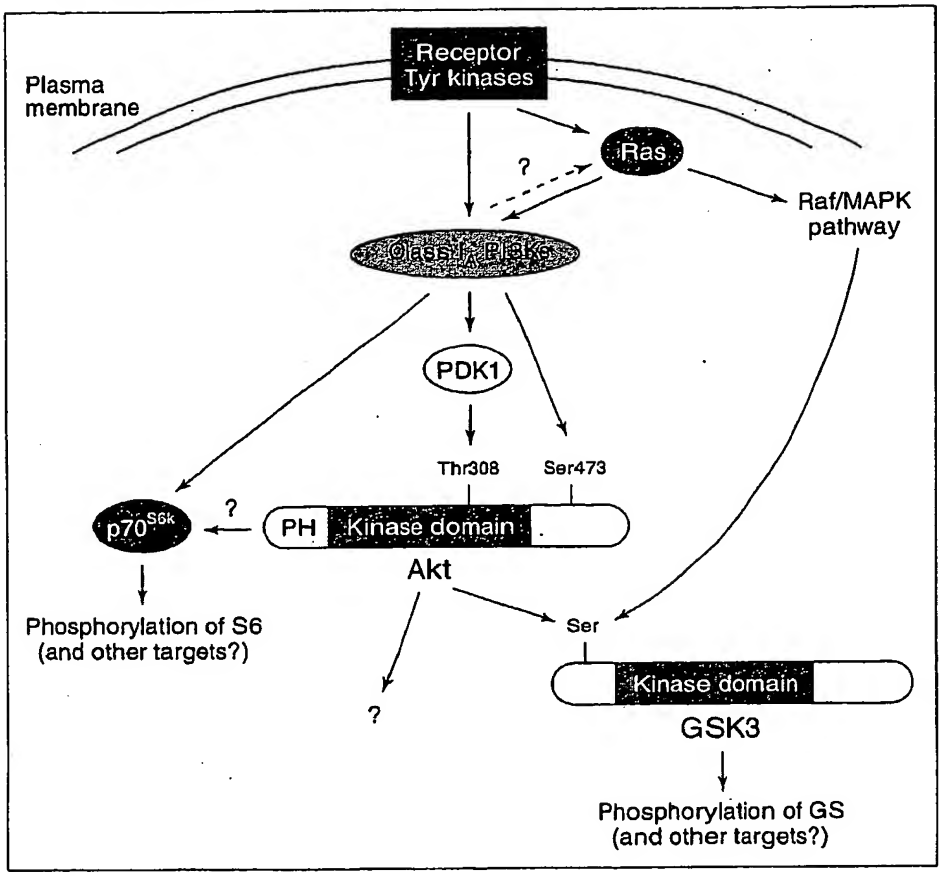


Figure 3

Signalling pathways linked to mammalian class I_A phosphoinositide 3-kinases. Arrows only denote a signalling pathway without specifying whether intermediary signalling molecules are involved. Abbreviations used: GSK3, glycogen synthase kinase-3; MAPK, mitogen-activated protein kinase; $p70^{S6k}$, p70 S6 kinase; PDK, PtdIns(3,4)P_2/(3,4,5)P_3-dependent kinase; PH, pleckstrin-homology domain.

(but not Ser473) *in vitro*[32]. Remarkably, the activity of this kinase is uniquely dependent on the presence of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2, and has therefore been named PtdIns(3,4)P_2/(3,4,5)P_3-dependent kinase-1 (PDK1).

Given that Akt is one of the most likely downstream targets of PI3Ks identified so far, and that Akt seems to play a central role in the PI3K-mediated protection against apoptosis[20], it is important to know what lies further downstream on this pathway (Fig. 3). There is some evidence to position $p70^{S6k}$ (see below) downstream of Akt[34]. To date, however, the only known substrate of Akt *in vivo* is glycogen synthase kinase-3 (GSK3). Upon stimulation of cells with insulin, Akt phosphorylates GSK3 on a single, conserved regulatory amino-terminal Ser in a PI3K-sensitive manner[35]. Phosphorylation and consequent inactivation of GSK3 results in the dephosphorylation and activation of a spectrum of metabolic and gene-regulatory proteins (reviewed in Ref. 36). Therefore, this link may turn out to be crucial for PI3K to exert its varied downstream effects. It should be mentioned that, in addition to PI3K and Akt, the Raf/mitogen-activated protein kinase (MAPK) pathway has also been implicated in GSK3 regulation by growth factors other than insulin (such as epidermal growth factor; reviewed in Ref. 36).

(2) $p70^{S6k}$ becomes activated upon mitogenic stimuli and plays an important role in the progression of cells from G1 to S phase of the cell cycle. It phosphorylates the S6 protein component of the 40S ribosomal subunit during mitogenic responses, but might also be involved in the regulation of other cellular processes (reviewed in Refs 37–39). The role of S6 phosphorylation is still not fully understood, but correlates with an increase in translation, probably from specific mRNAs encoding proteins essential for G1 progression (reviewed in Ref. 39).

Activation of $p70^{S6k}$ is regulated by multiple independent Ser/Thr-directed phosphorylations. This activation is independent of the Raf/MAPK pathway, but involves PI3Ks and the PIK-related kinase, mTOR (for mammalian-target of rapamycin), as well as PKC and as yet unidentified proline-directed kinases (reviewed in Refs 37–39). However, kinases that directly phosphorylate and activate $p70^{S6k}$ *in vivo* have not been identified.

(3) PKC/PRK. Both the lipid substrates and products of PI3Ks have been reported to activate, *in vitro*, a broad panel of PKC family members and PKC-related kinases [PRK, also indicated as protein kinases N (PKN)][40]. Although one group has shown that both PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 activate these kinases to the same extent, other groups (cited in Ref. 40) have reported conflicting data, which may be attributed to differences in lipid presentation procedures. Whereas PKCs may indeed be affected by PI3K lipid products, there is no consensus as to which member of this family, if any, is a selective target of these lipids.

Model systems in different organisms

In addition to mammals, PI3K genes have been identified in other organisms including yeasts, plants, slime molds, nematodes and fruit flies. Although the analysis of PI3K function in most of these organisms is at an early stage, future work applying genetic techniques should complement the mammalian studies in several respects. Most importantly, a genetic approach should allow the identification and characterisation of interacting genes, and improve our understanding of the function of different PI3Ks at the level of a cell, organ or entire organism. Below, we summarise the genetic systems currently emerging that are likely to play an important role in future studies of PI3K function.

Yeasts possess only class III PI3Ks and have already provided a useful model system to examine the function of this PI3K class. As described above, studies in *S. cerevisiae* and in mammalian cells have been complementary and suggest a ubiquitous role in constitutive vesicular trafficking[15].

The slime mold *D. discoideum* is a motile chemotactic unicellular organism that can form multicellular aggregates and fruiting bodies under conditions of nutritional stress. Four putative *D. discoideum* PI3K genes have been identified[41]. DdPIK1 and -2 resemble class I_A PI3Ks; DdPIK3 is most closely related to the class I_B group of G-protein-activated PI3Ks; and DdPIK5 closely resembles class III Vps34p-like PI3Ks. Separate disruption of each class I PI3K gene has no detectable phenotype whereas simultaneous disruption

of both class I_A PI3Ks has pleiotropic effects on growth and development, suggesting a certain level of redundancy[41]. By contrast, removal of the class III PI3K is lethal, as is removal of the class I_B PI3K together with either of the class I_A PI3Ks.

The nematode *C. elegans* is a more complex multicellular eukaryote with specialised terminally differentiated cells. *C. elegans* possesses genes encoding one PI3K from each class, though only the function of the class I_A PI3K homologue has been studied to date[42]. The class I_A PI3K gene, termed *age-1* or *daf-23*, was identified in genetic screens for mutants that promote longevity (*age* mutants)[43] and for mutants that affect dauer larvae formation (*daf* mutants)[44]. Under uncrowded conditions with ample food, wild-type *C. elegans* develop rapidly through four larval stages (L1–L4) to become adult worms with a life span of 2–3 weeks (Fig. 4). By contrast, when food is scarce and the population density high, an alternative third stage larva, the dauer larva, is formed. Dauer larvae are developmentally arrested, non-feeding and can endure harsh environmental conditions. When more favourable conditions return, dauers recover and develop into adults with a normal life span, irrespective of how much time has been spent as a dauer.

Null mutations in the *age-1* PI3K result in *C. elegans* that form dauer larvae constitutively, suggesting that *age-1* normally suppresses dauer formation under favourable conditions. Alternatively, *age-1* may be required for L3 development such that in the absence of *age-1* activity, dauer larva development occurs by default. Presumably, when nutrients are depleted, *age-1* is inactivated and an altered developmental programme that leads to dauer formation is initiated. *C. elegans* with reduced levels of the *age-1* PI3K (e.g. with maternal, but not zygotic *age-1* PI3K, or carrying weak mutant alleles of *age-1*) do not form dauer larvae, but develop into adults with significantly extended adult lifespans[45,48] (Fig. 4). A plausible explanation for this link between the daf and age phenotypes is that the genes normally expressed in the dauer larvae to make them long-lived and resistant to environmental stress are inappropriately expressed in adults with reduced levels of *age-1*, thereby conferring increased longevity and stress resistance[45,46].

The analysis of genetic interactions between *age-1* and various *daf* mutants has facilitated their ordering into a complex pathway affecting dauer formation and life span[47,48,53]. Characterisation of these genes at the molecular level should help to clarify the way in which the *age-1* PI3K functions as a signalling molecule. Mutations in *daf-2* also result in both constitutive dauer formation and increased adult longevity, while mutations in *daf-16* suppress both the dauer constitutive and increased longevity phenotypes resulting from mutations in *age-1* and *daf-2*. It will be intriguing to discover what the *daf-2* and *daf-16* genes encode.

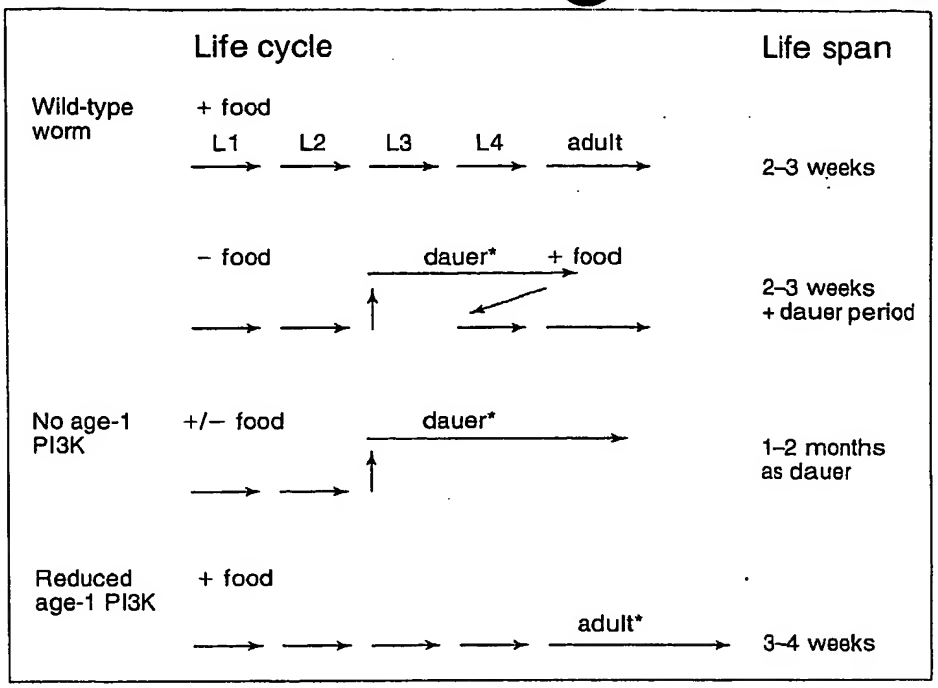


Figure 4
Mutations in age-1 phosphoinositide 3-kinase affect the life cycle of *Caenorhabditis elegans*. A scheme depicting the effects of loss of function mutations in the *age-1* PI3K gene on the nematode life cycle. The four larval stages (L1–L4) and the dauer larva, are shown. + food indicates that worms were growing in uncrowded conditions with ample food; – food indicates that worms were growing in over-crowded conditions with limited food; * indicates life-cycle stages with increased resistance to environmental stress.

The fruit fly *D. melanogaster* has genes encoding one PI3K from each class[14], so should also serve as a useful model system with which to address the function of the different PI3Ks. The *D. melanogaster* class I_A PI3K, Dp110 (Ref. 49; Table I) associates with p60, an SH2 domain-containing adaptor (Fig. 2), which, like its mammalian counterparts, recognises the pTyr-x-x-Met motif[50]. Ectopic expression studies have suggested that Dp110 might play a role in the control of cell growth[49]. The overproduction of Dp110 in wing or eye imaginal discs (sheaths of epithelial cells which expand and differentiate during larval growth and ultimately give rise to the structures that make up the adult fly) results in adult flies with enlarged wings or eyes, whereas overproduction of a dominant-negative version of Dp110 inhibits the growth of these structures[49]. Interestingly, loss of function mutations in the *D. melanogaster* homologue of the insulin receptor, Inr, also inhibits imaginal disc cell growth and result in the generation of smaller-than-wild-type flies[51]. So, in flies as well as mammals, class I_A PI3Ks might be important targets of the insulin receptor, and may regulate normal growth during development.

Concluding remarks

In recent years, a multitude of PI3Ks have been identified with unique molecular and biochemical characteristics indicating that they fall into distinct PI3K classes. The challenge now is to assign biological roles to each of these classes and to determine whether the lipids generated by the different classes of PI3Ks exert selective or redundant biological functions. One crucial issue is to find how 3-phosphorylated lipids mechanistically affect downstream signalling. To fully understand the biological events brought about by PI3K signalling, there is clearly a need to combine a variety of experimental approaches including pharmacology, biochemistry, cell biology and genetics.